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# CONTROLLED ATMOSPHERE STORAGE OF LETTUCE

by

D. K. Salunkhe
B. Singh
C. C. Yang
and
D. J. Wang

Utah State University
Department of Food Science and Industries
Logan, Utah 84321

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Studies were conducted on the effect of controlled atmosphere storage as well as chemical pretreatments on the quality of lettuce as well as biochemical composition of lettuce leaves, Lactuca sativa L., cultivar "Great Lakes."

In a CA of 2.5% $CO_2$ and 2.5% $O_2$, the lettuce heads could be stored up to 75 days. Pre-storage treatments with microbe- and senescence-inhibiting chemicals (Captan, Phaltan, Mycostatin and $N^6$-benzyladenine) had detrimental effects on the control atmosphere storage of lettuce at 35°F.

CA significantly inhibited the degradation of chlorophyll throughout the storage period. There was no significant effect of CA or other treatments on total sugars, reducing sugars, starch, total N, pH, organic acids, amino acids or total carotenes. The soluble proteins and the reducing sugars were lower in the CA lettuce than in the control lettuce. The lettuce treated with Plantan or Phaltan in combination with polyethylene packaging had higher amounts of soluble proteins and lower amounts of chlorophylls.


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## FOREWORD

Making available good quality fresh produce at overseas installations is an objective of the military. However, spoilage has been reported in lettuce shipped to military installations overseas. Thus each year the military sustains heavy losses to ship fresh produce overseas. Experience has shown that current handling practices do not dependably insure that fresh produce arriving in Asia will meet consumer standards. This can be expected when as much as eight weeks time may elapse between procurement and consumption. Causes for the spoilage included slime, decay, mold, and discoloration.

Laboratory investigations were conducted at the U.S. Army Natick laboratories on the effect of low oxygen controlled atmosphere system on the shelf life of lettuce. Results showed an advantage in favor of the controlled atmosphere system. This investigation was undertaken to identify one or more efficient procedures for the prolongation of shelf life of lettuce, so that good quality product can be delivered to military consumers overseas.

Project Officer and Alternate Project Officer for the U.S. Army Natick Laboratories, Plant Products Division, Food Laboratory were Dr. Abdul R. Rahman and Mr. Glenn Schafer, respectively. The work was conducted under Project 728012.12, Production Engineering.

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## ABSTRACT

Studies were conducted on the effect of controlled atmosphere storage as well as chemical pretreatments on the quality of lettuce as well as biochemical composition of lettuce leaves, Lactuca sativa L., cultivar "Great Lakes."

In a CA of 2.5% $CO_2$ and 2.5% $O_2$, the lettuce heads could be stored up to 75 days. Pre-storage treatments with microbe- and senescence-inhibiting chemicals (Captan, Phaltan, Mycostatin and $N^6$-benzyladenine) had detrimental effects on the control atmosphere storage of lettuce at 35°F.

CA significantly inhibited the degradation of chlorophyll throughout the storage period. There was no significant effect of CA or other treatments on total sugars, reducing sugars, starch, total N, pH, organic acids, amino acids or total carotenes. The soluble proteins and the reducing sugars were lower in he CA lettuce than in the control lettuce. The lettuce treated with Plantan or Phaltan in combination with polyethylene packaging had higher amounts of soluble proteins and lower amounts of chlorophylls.

PART I

EFFECTS ON QUALITY AND THE RESPIRATION RATE OF LETTUCE HEADS

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## INTRODUCTION

The effects of controlled atmosphere (CA), consisting of higher $CO_2$ and lower $O_2$ than normal air, on the shelf-life, quality, and chemical, physiological or biochemical processes of the fruits are well documented (Allen and Claypool, 1958; Allen and Smock, 1937; Fischer, 1942; Smock and Blanpied, 1958; Schomer and Olsen, 1964; Littlefield, 1968; Singh et al., 1970). Little is known, however, about its effects on leafy vegetables. McKenzie (1931) noted that as the $CO_2$ increased, there was a decrease in the rate of $CO_2$ evolution in lettuce. Thornton (1933) studied the effect of $CO_2$ storage on beans, cauliflower, celery, lettuce, and spinach. The response of the vegetables was found to vary with both temperature and $CO_2$ in the storage atmosphere (Thornton, 1931). Platenius (1943) found that lowering the $O_2$ concentration to 1.2% could reduce the respiration rate of asparagus by 55%. Leiberman and Hardenburg (1954) conducted experiments with broccoli and found that reduced $O_2$ concentration reduced the respiration rate. Recently, Isenberg and Sayles (1969) reported that storage of Danish cabbage under modified atmosphere reduced not only the losses due to respiration and trimming but the heads retained their green color, succulence, and texture.

The effect of CA on lettuce quality has been evaluated during actual (Stewart et al., 1970) and simulated shipment tests of about a week's duration (Lipton, 1967; Stewart and Uota, 1971; Watada et al., 1964). Recently, Rahman et al. (1970) determined the effects of low $O_2$ (Oxytrol®CA) for 22 days at 34-36° F. Lipton (1971) reported the effects of CA containing 21 or 3% oxygen combined with 0, 2, or 4% carbon dioxide on the market quality of lettuce stored for one month at 36 and 41° F. CA reduced decay particularly when low $O_2$ and high $CO_2$ were combined. Pink rib was inhibited by $CO_2$ and the yield of edible lettuce was higher after storage in CA than in air when stored at 36° F (Lipton, 1971).

This paper reports the effects on the quality and the respiration rate of lettuce of different combinations of gaseous concentrations ($CO_2$ and $O_2$), temperature, microbe- and senescence inhibiting chemicals in combination with CA and polyethylene packaging. A succeeeding paper presents the effect of CA and other treatments on the chemical composition of lettuce stored for 75 days.

## EXPERIMENTAL

In all the experiments to be described here, locally grown lettuce, cultivar 'Great Lakes', was used. The lettuce was commercially harvested and field packed (18 heads per carton) from parts of the field that appeared uniform. The packed cartons were immediately transferred to the refrigerated trailer and transported to the laboratory where CA chambers were located. Air-tight 50-gallon barrels with a humidity gauge on the cover were used as the storage containers. Wire metal screens separated the lettuce heads. Twenty-five heads of lettuce were placed in each barrel.

The concentrations of gases were maintained by a continuous flow of the appropriate mixtures of $O_2$, $CO_2$ and $N_2$ dispensed from pressure cylinders

(Fig. 1). A bottle of water and wet cheese cloth were used at the bottom of each barrel to maintain a high relative humidity in the container. The concentration of gas mixture was checked routinely with a "Fyrite" gas analyzer. The atmospheres in the control barrels were maintained by a continuous flow of air.

In order to determine the best gaseous combination and temperature for storage of lettuce in the first series of experiments, lettuce heads were stored at 35 and 70° F in the following controlled atmospheres: 1% $O_2$, 1% $CO_2$, 98% $N_2$; 2.5% $O_2$; 2.5% $CO_2$, 95% $N_2$; 5% $O_2$, 5% $CO_2$, 90% $N_2$; and 5% $O_2$, 95% $N_2$. The samples were taken on the 20th and 40th days of storage for quality evaluation.

Since the atmosphere containing 2.5% $O_2$ and 2.5% $CO_2$ at 35° F was found to be the best for prolonging shelf-life of lettuce, in subsequent experiments, lettuce was stored only at 35° F with 2.5% $O_2$ and 2.5% $CO_2$, and 95% $N_2$ (CA). In one series of experiments, before storage, each head was dipped separately for 5 minutes in one of the following water solutions: Captan$^R$ (N-trichloromethylthio)-4-cyclohexene-1,2-dicarbonimide), 100 ppm; Phaltan$^R$ (N-trichloromethylthiophthalimide), 1000 ppm; Mycostatin$^R$ (3-amino-3,6-dideoxy-D-aldohexose), 400 ppm; and $N^6$-benzyladenine, 20 ppm. In another set of three treatments $N^6$-benzyladenine, 20 ppm was used in combination with Captan$^R$, 1000 ppm; Phaltan$^R$, 1000 ppm; and Mycostatin$^R$, 400 ppm. In each treatment, Triton$^R$ B 1956, 1000 ppm was used as a surfactant. The control lettuce heads were treated with surfactant and stored at 35° F.

Phaltan$^R$ was better than the other chemicals in extending the shelf-life of lettuce. In one series of experiments, lettuce heads were treated with Phaltan$^R$, 1000 ppm and then stored with or without polyethylene packaging at 35° F and under 2.5% $CO_2$ and 2.5% $O_2$.

In the experiments to determine the best gaseous combination, chemical, and temperature for the storage of lettuce, the samples were taken from the barrels for quality evaluation on the 20th and 40th days of storage.

To determine lettuce quality and respiration rate after extended storage in CA of 2.5% $CO_2$ and 2.5% $O_2$ at 35° F, the samples were taken on the 15th, 30th, 40th, 60th, and 75th days. The sample sizes ranged from four to eight heads in each treatment.

## Quality evaluation

Visual observations for quality were made by a panel consisting of 6 to 10 persons. A subjective score rating was used according to the method described by El-Mansy *et al*. (1967) (See Table 1).

## Rate of respiration

The rates of $CO_2$ evolution of the whole lettuce head were measured

according to the method of Claypool and Keefer (1942). Oxygen uptake of the leaf discs was determined by the Gilson Respirometer. Discs, one cm in diameter, were cut by a steel cork borer from the leaves. The discs were rinsed with distilled water for 30 minutes. Twenty discs were blotted dry, quickly weighed, and placed in a Warburg vessel containing a total of 4.0 ml of solution which composition was 0.4 M sucrose, 0.05 M phosphate buffer pH 6.8 and 0.04 M KCL. After 15 minutes equilibration, the oxygen consumption at 70° F was determined at 20 minute intervals for periods up to two hours.

### Statistical analysis

The data were analyzed by analysis of variance and the means were compared by Tukey's $\omega$-procedure (Steel and Torrie, 1960).

## RESULTS

At 70° F, none of the concentrations of gases was beneficial. Lettuce could not hold more than 10 days at this temperature. Overall appearance was very poor, heads were discolored, tip burns appeared, and texture was poor and soft rot developed. The lettuce had pink ribs and butt discoloration. In addition, slime, molds, and other microorganisms appeared.

At 35° F, lettuce heads were field fresh, light green with no apparent sign of quality deterioration in all of the three combinations of gases up to 3 weeks (Table 2). When heads were examined after 40 days of storage, however, 2.5% $O_2$ and 2.5% $CO_2$ proved to be the best combination of gases for prolonging the shelf-life of lettuce (Table 2). In this concentration, lettuce heads were field fresh and had a bright green appearance. The heart leaves were sensitive to low-$O_2$ injury in the CA consisting of 1% $O_2$ and 1% $CO_2$; browning was evident after 40 days of storage at 35° F.

The effects of CA containing 2.5% $O_2$ and 2.5% $CO_2$ in combination with various chemicals on the quality are shown in Table 3. After 40-day storage, the lettuce heads treated with Phaltan$^R$ and Phaltan$^R$ + $N^6$-benzyladenine were better than those from other chemical treatments. It was noted that $N^6$-benzyladenine alone did not have any significant effect. In general, chemicals have detrimental effects on the quality of lettuce stored under CA at 35° F.

Table 4 presents the effects of CA (2.5% $CO_2$, and 2.5% $O_2$ at 35° F) and also of CA + Phaltan$^R$, 100 ppm; CA + Phaltan$^R$ + polyethylene packaging and CA + polyethylene packaging on the quality, dry weight, and the rate of respiration of lettuce 15, 30, 45, 60, and 75 days after storage. Compared to the controls (normal air and 35° F), CA-stored lettuce had significantly slower rate of respiration on the 15th, 30th, 45th, and 60th day of storage. The respiration rates of the CA-stored heads and the conventionally-stored heads were not significantly different on the 75th day of storage. This was true whether the rate of respiration was measured by $CO_2$-release with the Claypool and Keefer method or by $O_2$-uptake with a Gilson respirometer. The CA did not

have any significant effect on the dry weight of lettuce. The influence of CA on the quality of lettuce was not apparent up to the 30th day of storage. However, by the 45th day, the lettuce heads were better in quality when stored in CA. The differences in the quality were evident even on the 60th and 75th days of storage. Generally, lettuce stored in CA tasted sweeter and 95% of it was in salable condition at the end of the 75th day of storage.

The lettuce heads treated with Phaltan[R] or Phaltan[R] in combination with polyethylene packing before storage in CA had a higher rate of respiration and poorer quality than the CA- or conventionally-stored lettuce.

## DISCUSSION

Our results indicate that temperature is of primary importance in keeping up the quality of lettuce. It agrees with the results of the others (Watada et al., 1964; Lipton and Barger, 1965; Harvey, 1969). Nevertheless the added advantage of controlled atmosphere was quite apparent. The lettuce stored at $35^{\circ}$ F and 2.5% $CO_2$ and 2.5% $O_2$ maintained its quality better than that kept at the same temperature but in the normal air for a period of 75 days. This was in contrast to the results obtained by Watada et al. (1964), where no noticeable effects were evident when lettuce heads were stored for 4-8 days at $41^{\circ}$ F in CA of 2.5% $CO_2$ and 2.5% $O_2$. Apparently the differences in the results were caused by the very short storage time and the higher temperature. We have noted that beneficial effects of CA were more pronounced and clearly evident after 39 days of storage.

The chief benefits of CA storage are the reduction in decay, pink rib and butt discoloration, and russet spotting. In all combinations, the atmosphere containing lower $O_2$ was more beneficial than air for reducing russet spotting. This phenomenon has also been observed by Parsons et al. (1964), Lipton (1967), and Stewart et al. (1970). The incidence of pink mid rib and butt discoloration appeared to be more serious in lettuce conventionally refrigerated storage than that held in CA. Rather similar results were obtained by Stewart et al. (1970) and Lipton (1967). According to Lipton (1970), pink rib was reduced in atmospheres with added $CO_2$. Low-$O_2$ either exerted no effect or slightly aggravated the disorder in lettuce stored for 1 month. Stewart and Uota (1971) did not find any effect of $CO_2$ on pink rib during the shorter period of sotrage. The heart leaves were sensitive to low-$O_2$ injury in the CA of 1% $O_2$ and 1% $CO_2$.

Under the conditions in which we conducted our experiments, all chemicals had a significantly deterimental effect on the shelf-life and quality of lettuce. $N^6$-benzyladenine (a senescence-inhibiting chemical) had been reported to have a beneficial effect on several types of fresh produce. For example, Bessey (1960) found that lettuce heads treated with $N^6$-benzyladenine were maintained in fresh green condition considerably longer than untreated heads. It was also effective in delaying the visual manifestation of senescence in lettuce, endive, Brussels sprouts, broccoli, mustard greens, radish tops, parsley, celery, green onions, and asparagus (Zink 1961). These results were further substantiated by

Lipton and Ceponis (1962) and El-Mansy et al. (1967) in the case of lettuce, and Salunkhe et al. (1962) in the case of cauliflower, endive, parsley, snap-beans, and lettuce. In contrast to the aforementioned effects of $N^6$-benzyladenine, we did not encounter any beneficial effect of its use on the shelf-life of lettuce.

The fungicides, Mycostatin[R] and Captan[R] had detrimental effects on the shelf-life and the quality of lettuce although these have been reported to have beneficial effects on sweet cherries (Do et al., 1966) and on lettuce (Salunkhe and Norton, 1960). Probably, the effects of fungicides and senescence inhibitors are manifested only under unfavorable holding conditions such as long-time storage at undesirably high holding temperatures. (Lipton and Ceponis, 1962).

The rate of respiration whether measured by $CO_2$ evolution or $O_2$ uptake was reduced by CA (2.5% $CO_2$ and 2.5% $O_2$). The data presented in this paper do not represent the value for respiration of the roots during storage. It is evident, however, that the high $CO_2$ concentration inhibits respiratory rate which could even be observed after removal of the heads from storage. The residual effect may well be due either to a high concentration of $CO_2$ left out in the cells or a permanent effect on the cell organelles (such as mitochondria) or enzyme protein (conformation) of the lettuce stored under CA. The beneficial effects of CA have been attributed to its inhibition of the respiration rate. This is in conformity with the studies of McKenzie (1931) on lettuce; Claypool and Allen (1948) on apricots, grapes, peaches, pears, and plums; Lieberman and Hardenburg et al. (1958) on broccoli; Singh et al. (1970) on sweet cherries; and Karnik et al. (1970) on sugar beets. It has been suggested that CA affects the activities of enzymes concerned with $CO_2$ evolution in respiration. These may include malic enzyme and pyruvate decarboxylase (Littlefield, 1968). Bonner (1950) and Ranson et al, (1957) reported that increased $CO_2$ concentration inhibited the activities of several respiratory enzymes. Frenkel and Patterson (1969) observed that in pears, higher $CO_2$ concentrations (5 to 20%) inhibited succinic dehydrogenase. The detrimental effects of chemicals and packaging appeared to be due to the increased rates of respiration noted during the storage in the present study.

The moisture content and the weight of the lettuce during 75 days of storage in all treatments did not change significantly even at the end of the storage. This may have resulted from the high relative humidity maintained by a bottle of water and a wet cheese cloth at the bottom of each barrel.

Table 1. Description of numerical quality ratings used to evaluate 'Great Lakes' lettuce.

| Numerical rating[a] | Descriptive rating | Visual observations |
|---|---|---|
| 9 | Excellent | Field fresh, bright green appearance, and free from defects. |
| 7 | Good | Minor defects present but not objectionable. Green color slightly bleached and dull. Good retail sales appeal. |
| 5 | Fair | Outer leaves showing slight yellowing. Could be returned to acceptable condition with slight trimming. |
| 3 | Unsalable | Slime and other defects serious; would not be eaten. |
| 1 | Unusable | Slime and other defects serious; would not be eaten. |

[a]Intermediate scores used when appropriate.

Table 2. Effects of different combinations of $CO_2$ and $O_2$ storage on the quality of lettuce at 35° F and 95 per cent RH.[a]

| Days after storage | Conc. of gases % $O_2$ | Conc. of gases % $CO_2$ | Numerical rating | Descriptive rating | Visual observation |
|---|---|---|---|---|---|
| 20 | 21 | <1 | 9 | Excellent | Generally, lettuce stored in these atmospheres had a fresh, green appearance, no defects, except lettuce held in 1:1= $O_2$:$CO_2$ mixture had slight yellowing on the tip of outer leaves |
| | 5 | 5 | $8^{*}$ | Excellent | |
| | 5 | 0 | $9^{ns}$ | Excellent | |
| | 2.5 | 2.5 | $9^{ns}$ | Excellent | |
| | 1 | <1 | $8^{*}$ | Excellent | |
| 40 | 21 | 1 | 7 | Good | Minor defects only, red butt |
| | 5 | 5 | $5^{**}$ | Fair | Outer leaves having brown spotting, red butt |
| | 5 | 0 | $7^{ns}$ | Good | Minor defects |
| | 2.5 | 2.5 | $8^{*}$ | Excellent | Field fresh, bright green |
| | 1 | 1 | $5^{**}$ | Fair | Red heart leaves, reddish midrib, soft in texture |

[a]Analysis of variance and comparison of means by Tukey's ω-procedure.
*Significantly different from control at 0.05 level.
**Significantly different from control at 0.01 level.
[ns]Not significantly different from control.

Table 3. Effects of chemical treatments on lettuce stored in 2.5 per cent $O_2$ and 2.5 per cent $CO_2$ at $35^{o}$ F and 95 per cent RH for 40 days.[a]

| Treatment | | Numerical rating | Descriptive rating | Visual observation |
|---|---|---|---|---|
| Chemical | Conc. (ppm) | | | |
| Control | | 8.5 | Good | Minor defects, red butt |
| Captan | 1,000 | 5.0** | Fair | Slight yellowing on outer leaves |
| Phaltan | 1,000 | 7.0* | Good | Minor defects |
| Mycostatin | 400 | 6.5** | Fair | Little red spotting, and midrib |
| $N^6$-BA[b] | 20 | 5.0** | Fair | Slight yellowing, red midrib on the outer leaves |
| Captan + $N^6$-BA | 1,000<br>20 | 5.0** | Fair | Slight red rib and yellowing |
| Phaltan + $N^6$-BA | 1,000<br>20 | 7.0* | Good | Minor defects |
| Mycostatin + $N^6$-BA | 400<br>20 | 5.5** | Fair | Slight yellowing, and red rib on the wrapper leaves |

[a]Analysis of variance and comparison of means by Tukey's ω-procedure.
[b]$N^6$-benzyladenine.
*Significantly different from control at 0.05 level.
**Significantly different from control at 0.01 level.

Table 4. Physiochemical changes in lettuce during controlled atmosphere storage (2.5% $O_2$, 2.5% $CO_2$) at 35°F in relation to treatments.[a]

| Days of storage | Treatment | Dry[b] Weight (%) | $CO_2$[c] release mg/hr kg | $O_2$[c] uptake mg/hr kg | Quality[d] rating |
|---|---|---|---|---|---|
| 0 | Control | 4.9 | 35.1 | 176.1 | 9 |
| 15 | Control | 5.0 | 25.5 | 170.0 | 9 |
| | CA | 4.7 | 21.5* | 152.4* | 9$^{ns}$ |
| | CA + Ph[e] | 4.7 | 29.3* | 238.1* | 9$^{ns}$ |
| | CA+Ph+Pkg[e] | 4.3 | 28.0* | 260.7* | 9$^{ns}$ |
| | CA + Pkg | 4.6 | 21.3* | 135.3* | 9$^{ns}$ |
| 30 | Control | 4.9 | 19.8 | 135.5 | 9 |
| | CA | 4.7 | 15.0* | 104.4* | 9$^{ns}$ |
| | CA + Ph | 4.7 | 23.8* | 202.6* | 7* |
| | CA+Ph+Pkg | 4.5 | 22.6* | 186.5* | 7* |
| | CA + Pkg | 4.7 | 17.0* | 118.7* | 9$^{ns}$ |
| 45 | Control | 4.9 | 34.96 | 171.4 | 8 |
| | CA | 4.7 | 30.19* | 152.0* | 9* |
| | CA + Ph | 4.7 | 45.45* | 275.1* | 5** |
| | CA+Ph+Pkg | 4.7 | 40.20* | 243.9* | 5** |
| | CA + Pkg | 4.8 | 32.16$^{ns}$ | 167.4$^{ns}$ | 7* |
| 60 | Control | 4.4 | 31.69 | 210.7 | 7 |
| | CA | 3.7 | 26.95* | 183.0* | 8* |
| | CA + Pkg | 3.3 | 27.25* | 188.2* | 5** |
| 75 | Control | 4.2 | 10.65 | 188.2 | 5 |
| | CA | 3.4 | 10.17$^{ns}$ | 175.3$^{ns}$ | 7** |

[a]Analysis of variance and comparison of means by Tukey's ω-procedure
[b]Results expressed on fresh weight basis.
[c]Results expressed on dry weight basis.
[d]8, 9 = excellent; 6, 7 = good; 4, 5 = fair; 2, 3 = unsalable; 1 = unusable
[e]Ph = Phaltan; Pkg = polyethylene packaging.
*Significantly different at 0.05 level, **at 0.01 level, and $^{ns}$not significantly different at 0.05 from control.

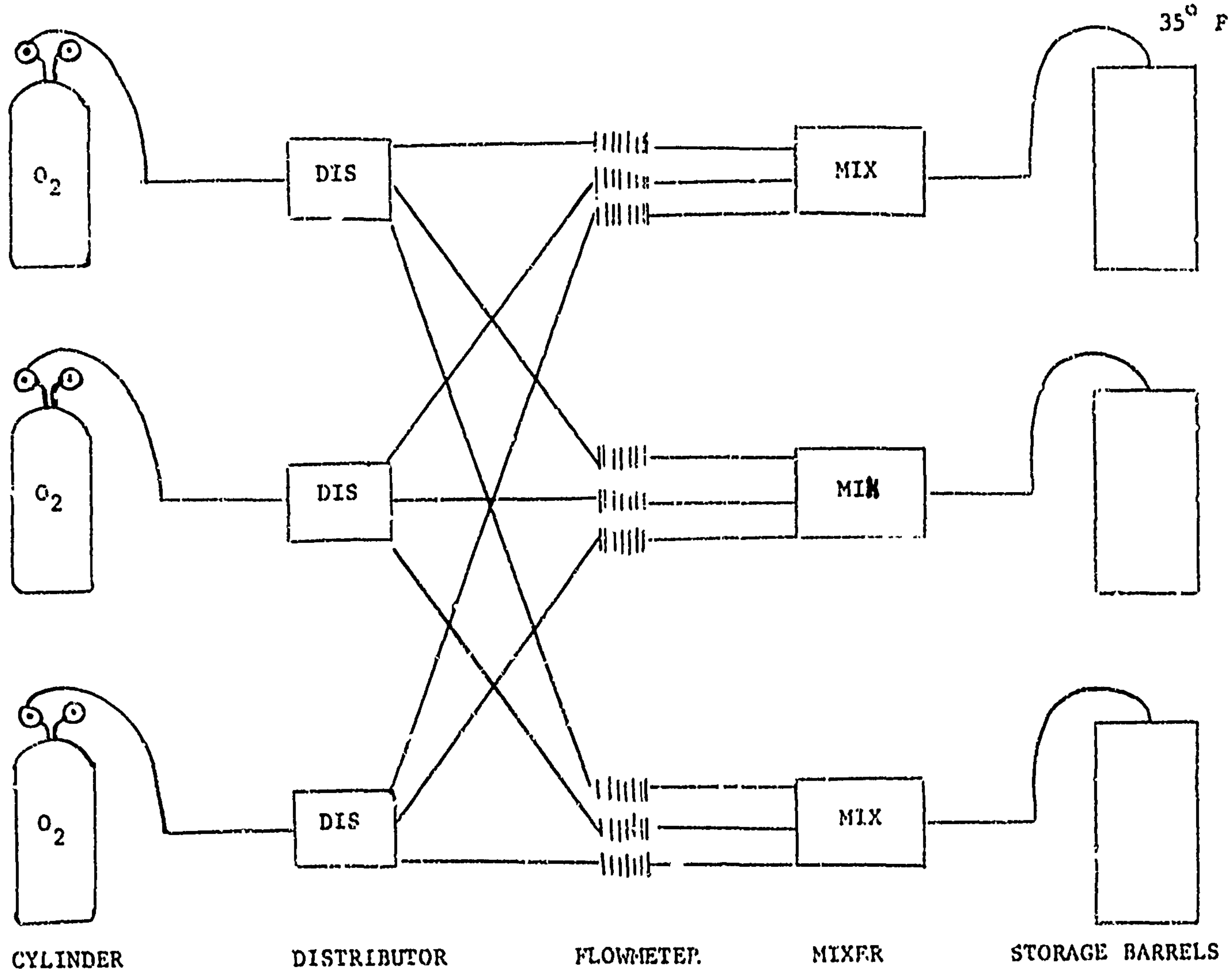


Fig. 1. Schematic diagram of controlled atmosphere storage facilities.

## Summary

Lettuce, Lactuca sativa L., cultivar 'Great Lakes' was stored at 35 and 70° F and 90-95% relative humidity in one of the following atmospheres: (1) 5% $CO_2$ and 95% $N_2$; (2) 5% $O_2$ and 95% $N_2$; (3) 1% $CO_2$, 1% $O_2$, and 98% $N_2$; (4) 2.5% $CO_2$, 2.5% $O_2$, and 95% $N_2$. The quality of lettuce was evaluated on the 20th and 40th day of storage. At 35° F, lettuce heads could be stored up to 3 weeks without much quality deterioration in any one of the gaseous combinations except 5% $CO_2$. In a CA of 2.5% $CO_2$ and 2.5% $O_2$, the lettuce heads could be stored up to 75 days. Pre-storage treatments with microbe- or senescence-inhibiting chemicals (Captan®, 1000 ppm; Phaltan®, 1000 ppm; Mycostatin®, 400 ppm; and $N^6$-benzyladenine, 20 ppm) had deterimental effects on the controlled atmosphere storage of lettuce at 35° F. CA (2.5% $CO_2$ and 2.5% $O_2$) and CA combined with polyethylene packaging reduced the rate of respiration while CA combined with Phaltan® (1000 ppm) enhanced the rate of respiration of the lettuce. The dry weight or the moisture content of the lettuce was not affected by CA or any other treatments under the experimental conditions.

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# PART II

# EFFECTS ON BIOCHEMICAL COMPOSITION OF THE LEAVES

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## Introduction

In a preceding paper (Singh et al., 1971), we described the effects of CA and CA in combination with chemicals and packaging on the quality and respiration rate of lettuce. In this paper, we report the biochemical changes occurring in the leaves of lettuce during storage under CA (2.5% $CO_2$ and 2.5% $O_2$) alone and in combination with Phaltan[R] treatment and polyethylene packaging.

The effects of CA on the biochemical composition of several fruits and vegetables have been investigated. CA delays the rate of degradation of chlorophyll in spinach (McGill et al., 1966), green beans (Groeschel et al., 1966), broccoli (Lebermann et al., 1968), and asparagus (Wang et al., 1971). Wankier et al. (1970) noted that CA slowed down the degreening process in apricots and peaches. A marked increase in pH has been noted in green beans (Groeschel et al., 1966), spinach (Brugheimer et al., 1967), broccoli (Lebermann et al., 1968), and asparagus (Wang et al., 1971) stored in CA. Thornton (1933) reported that treatment of fruits, roots, stems, tubers, bulbs, and even entire potted plants with $CO_2$ in the presence of $O_2$ caused increased pH of sap extracted from the tissues. The increase in pH was paralleled by a decrease in titratable acidity in beans (Groeschel et al., 1966), spinach (Burgheimer et al., 1967), broccoli (Lebermann et al., 1968), and asparagus (Wang et al., 1971).

Ranson (1953) observed no major changes in the acid content of Kalanchoe leaves (12 hours), carrot roots (3 days), and oat coleoptiles (1 day) after storage in atmospheres containing less than 10% $CO_2$. In atmospheres containing 20 to 90% $CO_2$, succinate and aminobutyrate accumulated; while malate, aspartate, and alanine were depleted. Littlefield (1968) noted that leucine, valine, threonine, and aspartic and malic acid were more in concentration in apples and pears stored in CA than those stored in the normal air. In CA-stored apricots and peaches, the concentration of succinic acid was higher and that of malic and citric acids was lower than the controls. Singh et al. (1970) found that "Lambert" sweet cherries stored at higher $CO_2$ concentrations had a lower amount of tyrosine and higher amounts of $\alpha$-aminobutyric and malic acids than fruits stored in the conventional refrigerator. Hulme (1956) reported an accumulation of succinate in plants stored in atmospheres containing more than 20% $CO_2$. Ranson et al. (1957) suggested that the accumulation of succinate was due to succinic oxidase sensitivity to higher levels of $CO_2$. Frenkel and Patterson (1969) found that the activity of succinic dehydrogenase was inhibited if the pears were kept at $CO_2$ concentrations above 0.03%. Karnik et al. (1970b) noted that citric and succinic acid accumulated in sugarbeet roots stored in CA.

The effects of CA with respect to sugars and starch vary. In apples, pears, cherries, peaches, and "Moorpack" apricots, the total or reducing sugars were unaffected by CA (Littlefield, 1968; Singh et al., 1970; Wankier et al., 1970); while in "Large Early Montgamet" apricots, there were less reducing sugars (Wankier et al., 1970). Karnik et al. (1970a, 1970b) found that the roots of sugar beets stored in CA contained more sucrose and less

raffinose and reducing sugars. McGill *et al.* (1966) did not find any definite trend in the contents of starch in spinach stored in CA.

Harris and Von Loesuche (1960) reported that CA caused an increase in tissue protein, retarded sugar loss, increased loss of reduced ascorbic acid, and retarded changes in pectic substances in fruits. Wankier *et al.* (1970) pointed out that CA-stored apricot and peach fruits retained a higher concentration of total pectins than did conventional refrigerator stored fruits. Li (1963) noted that in pears, total alcohol soluble nitrogen and protein nitrogen decreased at a slower rate in CA, resulting in higher concentrations at the end of the storage period. Groeschel *et al.* (1966) found more soluble nitrogen in the conventional refrigeration than in the samples of beans stored in CA.

## Experimental

The source of lettuce and the arrangements for CA were essentially the same as described in Part I (Singh *et al.*, 1971). Each of the following 4 groups of lettuce was maintained at 2.5% $CO_2$ and 2.5% $O_2$ at 35° F and 95% relative humidity. Group 5 was maintained under normal air at 35° F.

Group 1 - Lettuce without any treatment stored in 2.5% $CO_2$ and 2.5% $O_2$ (CA).

Group 2 - Lettuce was treated with 1000 ppm Phaltan^R + 1000 ppm Triton-B 1956 (CA + Ph).

Group 3 - Lettuce was treated with 1000 ppm Phaltan^R + 1000 ppm Triton-B 1956 (CA + Ph + Pkg) and then packed in polyethylene bags.

Group 4 - Lettuce was packed in polyethylene bags without any chemical treatment (CA + Pkg).

Group 5 - Lettuce without any treatment stored under normal air (conventional refrigeration).

The samples for analyses were taken after 15, 30, 45, 60, and 75 days of storage. For the determination of pH and titratable acidity, the leaves were homogenized in a Waring Blender. The homogenate was filtered through four layers of cheese cloth. pH was determined with a Beckman Expandomatic SS-2 model pH meter. The glass-electrode method described by AOAC (1960) was used to measure titratable acidity. Chlorophyll a and b were determined according to the AOAC (1960).

Freeze-dried powder of the leaves was used to determine total nitrogen soluble protein, amino acids, organic acids, total and reducing sugars, starch, and total carotenes. Total nitrogen was estimated by the standard

micro-Kjeldahl method. Soluble proteins were extracted with water and then determined by the Folin-Phenol reagent method of Lowry et al. (1951). A modified ninhydrin colorimetric analysis was used for free amino acid determination (Rosen, 1957) of the ethanol extracts of the leaves. The method of Williams (1961) was employed to measure organic acids and the method of Nelson (1944) was used to determine reducing sugars. Starch and total sugars were extracted and measured by the method of McCready et al. (1950). AOAC standards were used to measure the total carotenes.

Analysis of variance with a split plot technique was used to analyze the data. The means were compared by Tukey's ω-procedure (Steel and Torrie, 1960).

## Results and Discussion

Compared to the controls, chlorophyll retention of the lettuce was significantly higher in CA and CA combined with packaging (Table 1, Fig. 1), but was lower in lettuce treated with Phaltan$^R$. A similar effect has been observed (Groeschel et al., 1966; Lebermann et al., 1968; Wankier et al., 1970; Wang et al., 1971). Wang et al. (1971) suggested a positive correlation between the amounts of chlorophyll and the changes of pH. Groeschell et al. (1966) also noted that the better retention of chlorophyll in the CA-stored sample was always accompanied by a higher pH than in the air-stored sample. The results of the present experiments indicate that there was no significant increase in the pH of the lettuce stored in CA. In CA-stored lettuce, the titratable acidity was lower than the control till the 30th day of storage. On the 45th day, the differences between the CA and the controls were not significant; and by the 60th day, the titratable acidity became higher than the control and remained higher up to the end of the storage period. Storage experiments have been conducted for most of the vegetables for short durations. It has been noted that titratable acidity decreases in the CA-stored samples (Groeschel et al., 1966; Burgheimer et al., 1967; Lebermann et al., 1968; and Wang et al., 1971). Compared to the controls, the titratable acidity was higher in lettuce stored 30 days or longer in CA with pre-storage treatments of Phaltan$^R$, packaging, and Phaltan$^R$ with packaging. This is consistent with the chlorophyll retention data in these treatments, which was lower than the controls.

The total protein values were calculated from the total N (total N x 6.25). Changes in total protein were not significant in the CA (Table 3) in any of the treatments. However, the soluble proteins of lettuce were significantly lower in CA and CA combined with packaging throughout the storage period (Table 3, Fig. 3). The lettuce treated with Phaltan$^R$ and Phaltan$^R$ combined with packaging had higher content of soluble proteins by the 45th day of storage when it started to decay.

Although starch and total sugars of all treatments decreased during storage, CA and CA in combination with packaging showed a higher retention of starch and total sugars throughout the storage period than the conventional refrigeration (Table 2, Fig. 2). Lettuce treated with Phaltan$^R$ and Phaltan$^R$

combined with packaging, on the contrary, showed lower starch and total sugars than that of conventional refrigeration. The statistical analysis, however, indicated significantly lower total sugars only on the 15th and the 30th day of storage in Phaltan$^R$-treated lettuce. This is consistent with the stimulatory effects of Phaltan$^R$ on the rate of respiration of lettuce reported in Part I (Singh et al., 1971). The nonsignificant changes in starch and total sugars noted in these experiments concur with those of McGill et al. (1966) for spinach, Wankier et al. (1970) for peaches and "Moorpack" arpicots, and Singh et al. (1970) for cherries. Although the lettuce in CA and CA with packaging and pre-storage treatment with Phaltan$^R$ and Phaltan$^R$ combined with packaging had lower reducing sugars than that of conventional refrigeration throughout the period of storage, the differences were only significant in CA and CA combined with packaging on the 60th and 75th day of storage. Wankier et al. (1970) and Karnik et al. (1970 a, 1970b) also found a lower amount of reducing sugars in CA-stored apricots, peaches, and sugar beets.

Compared to the conventional refrigeration storage, the total organic acids (expressed as malic acid) in CA and CA in combination with packaging were lower on the 15th, 30th, and 45th days. After 60 days, however, lettuce in CA with packaging showed 5% higher and lettuce in CA alone 6% higher total organic acid content than the conventional refrigeration (Fig. 3, Table 3). Lettuce treated with Phaltan$^R$ and Phaltan$^R$ combined with packaging showed a lower organic acid content on the 15th day, but it increased rapidly on the 30th day of storage; and it was 10% higher than conventional refrigeration on the 45th day of storage. The statistical data, however, indicated that in most cases, the differences in the contents of organic acids were nonsignificant. The accumulation of organic acids in CA-stored samples has been noted by several investigators (Ranson et al., 1956; Littlefield, 1968; Singh et al., 1970; Wankier et al., 1970; Karnik et al., 1970). It is generally considered that the accumulation of organic acids is caused by the slower utilization rate of TCA cycle acids of the fruits and vegetables stored in CA. This was supported by Frankel and Patterson (1969), who found that higher $CO_2$ concentrations inhibited succinic acid dehydrogenase in pears.

There was no significant difference between the content of total free amino acids of the lettuce stored in CA and that stored in conventional refrigeration. Lettuce from CA combined with packaging showed a higher amount of amino acids on the 60th day. The amino acids were lower on the 15th day but were not significantly affected on the subsequent days in lettuce stored under CA with pre-storage treatment of Phaltan$^R$. It appears that effects of CA on amino acids vary according to the species. For example, Littlefield (1968) found that amino acids in general were higher in pears and apples stored in CA. Singh et al. (1970) reported that sweet cherries stored in CA had a lower amount of tyrosine and higher amounts of α-aminobutyric acid than fruits stored in the conventional refrigerator. In the roots of sugar beets stored in CA, amino acids were smaller in amount than those stored in conventional refrigeration (Karnik et al., 1970).

Although none of the treatments affected significantly the concentration of total carotenes, the content of carotenes was 10% higher in the CA and CA

combined with packaging than the conventional refrigeration on the 60th and 75th day of storage (Table 1, Fig. 4). This may be due to a lower rate of carotene destruction by lipoxidase in the lettuce stored under these conditions Compared to the conventional refrigeration, total carotenes in lettuce treated with Phaltan$^{R}$ and Phaltan$^{R}$ combined with packaging decreased after 30 days of storage.

**Table 1. Effects of controlled atmosphere (CA) and CA in combination with Phaltan and polyethylene packaging on pH, titratable acidity, chlorophyll, and total carotenes of lettuce.[a]**

| Day of storage | Treat- ment | pH | Titratable acidity me acid/g | Chlorophyll mg/g | | | Total carotenes µg/g |
|---|---|---|---|---|---|---|---|
| | | | | a | b | Total | |
| 0 | CR | 6.41 | 0.1678 | 35.5 | 18.9 | 54.6 | 33.39 |
| 15 | CR | 6.35 | 0.1695 | 20.5 | 12.5 | 33.3 | 22.95 |
| | CA | 6.42$^{ns}$ | 0.1610$^{**}$ | 20.5$^{ns}$ | 12.9$^{ns}$ | 33.5$^{ns}$ | 25.52$^{ns}$ |
| | CPh | 6.36$^{ns}$ | 0.1680$^{ns}$ | 19.6$^{ns}$ | 12.4$^{ns}$ | 31.9$^{ns}$ | 25.96$^{ns}$ |
| | CPP | 6.43$^{ns}$ | 0.1513$^{**}$ | 20.0$^{ns}$ | 12.1$^{ns}$ | 32.1$^{ns}$ | 25.28$^{ns}$ |
| | CPk | 6.42$^{ns}$ | 0.1581$^{**}$ | 20.9$^{ns}$ | 12.9$^{ns}$ | 33.9$^{ns}$ | 25.91$^{ns}$ |
| 30 | CR | 6.40 | 0.1712 | 26.2 | 14.6 | 40.8 | 24.79 |
| | CA | 6.41$^{ns}$ | 0.1617$^{**}$ | 17.6$^{*}$ | 15.2$^{ns}$ | 42.8$^{ns}$ | 27.00$^{ns}$ |
| | CPh | 6.37$^{ns}$ | 0.1832$^{**}$ | 26.7$^{*}$ | 12.7$^{ns}$ | 39.4$^{ns}$ | 29.95$^{ns}$ |
| | CPP | 6.33$^{ns}$ | 0.1798$^{**}$ | 24.1$^{ns}$ | 13.1$^{ns}$ | 37.2$^{*}$ | 30.78$^{*}$ |
| | CPk | 6.42$^{ns}$ | 0.1746$^{**}$ | 27.3$^{ns}$ | 15.1$^{ns}$ | 42.4$^{ns}$ | 27.06$^{ns}$ |
| 45 | CR | 6.47 | 0.1723 | 28.3 | 16.9 | 45.2 | 18.49 |
| | CA | 6.49$^{ns}$ | 0.1699$^{ns}$ | 30.4$^{ns}$ | 17.7$^{ns}$ | 48.1$^{ns}$ | 20.51$^{ns}$ |
| | CPh | 6.30$^{ns}$ | 0.2015$^{**}$ | 23.7$^{*}$ | 14.7$^{*}$ | 38.4$^{*}$ | 18.50$^{ns}$ |
| | CPP | 6.15$^{**}$ | 0.2033$^{**}$ | 24.1$^{*}$ | 14.3$^{*}$ | 38.4$^{*}$ | 18.17$^{ns}$ |
| | CPk | 6.44$^{ns}$ | 0.1775$^{**}$ | 29.7$^{ns}$ | 17.5$^{ns}$ | 47.2$^{ns}$ | 19.57$^{ns}$ |
| 60 | CR | 6.45 | 0.1725 | 31.9 | 17.7 | 49.7 | 18.41 |
| | CA | 6.42$^{ns}$ | 0.1776$^{**}$ | 34.5$^{ns}$ | 18.8$^{ns}$ | 53.3$^{*}$ | 20.46$^{ns}$ |
| | CPk | 6.47$^{ns}$ | 0.1802$^{**}$ | 34.6$^{ns}$ | 19.3$^{ns}$ | 53.9$^{*}$ | 20.34$^{ns}$ |
| 75 | CR | 6.40 | 0.1764 | 31.2 | 20.3 | 51.5 | 26.34 |
| | CA | 6.41$^{ns}$ | 0.1979$^{**}$ | 33.9$^{ns}$ | 22.2$^{ns}$ | 56.0$^{ns}$ | 29.00$^{ns}$ |

a: Results expressed on dry weight basis

CR: Conventional refrigeration; CA: Controlled atmosphere (2.5% $O_2$ and 2.5% $CO_2$)

CPh: CA + Phaltan (1,000 ppm). CPP: CA + Phaltan (1,000 ppm) + packaging in polyethylene bags.

CPk: CA + packaging in polyethylene bags.

me: Milliequivalent weight

* Significant at 0.05 level; ** Significant at 0.01 level.

ns Not significant at 0.05 level compared to controls.

Table 2. Effects of controlled atmosphere (CA) and CA in combination with Phaltan and polyethylene packaging on total starch, total sugars, and reducing sugars of lettuce.[a]

| Day of storage | Treatment | Total starch mg/g | Total sugars mg/g | Reducing sugars mg/g |
|---|---|---|---|---|
| 0 | CR | 5.38 | 132.70 | 49.36 |
| 15 | CR | 4.81 | 100.72 | 51.90 |
| | CA | 5.09$^{ns}$ | 103.24$^{ns}$ | 48.41$^{ns}$ |
| | CPh | 4.04$^{ns}$ | 83.08$^{*}$ | 39.67$^{**}$ |
| | CPP | 4.55$^{ns}$ | 90.04$^{ns}$ | 39.55$^{**}$ |
| | CPk | 5.25$^{ns}$ | 102.61$^{ns}$ | 46.44$^{ns}$ |
| 30 | CR | 4.05 | 103.34 | 57.15 |
| | CA | 4.07$^{ns}$ | 96.18$^{ns}$ | 46.29$^{ns}$ |
| | CPh | 4.08$^{ns}$ | 35.88$^{*}$ | 42.63$^{*}$ |
| | CPP | 4.15$^{ns}$ | 99.14$^{ns}$ | 44.42$^{*}$ |
| | CPk | 4.40$^{ns}$ | 109.79$^{ns}$ | 52.60$^{ns}$ |
| 45 | CR | 2.67 | 69.16 | 53.95 |
| | CA | 2.61$^{ns}$ | 68.73$^{ns}$ | 46.48$^{ns}$ |
| | CPh | 2.67$^{ns}$ | 63.81$^{ns}$ | 38.99$^{**}$ |
| | CPP | 2.95$^{ns}$ | 65.77$^{ns}$ | 40.96$^{**}$ |
| | CPk | 2.72$^{ns}$ | 79.56$^{**}$ | 50.24$^{ns}$ |
| 60 | CR | 2.81 | 85.02 | 55.67 |
| | CA | 2.90$^{ns}$ | 87.43$^{ns}$ | 48.99$^{*}$ |
| | CPk | 3.33$^{*}$ | 86.40$^{ns}$ | 46.80$^{**}$ |
| 75 | CR | 2.64 | 77.99 | 50.06 |
| | CA | 2.64$^{ns}$ | 83.47$^{ns}$ | 45.56$^{*}$ |

a: Results expressed on dry weight basis

CR: Conventional refrigeration; CA: controlled atmosphere (2.5% $O_2$ and 2.5% $CO_2$).

CPh: CA + Phaltan (1,000 ppm). CPP: CA + Phaltan (1,000 ppm) + packaging in polyethylene bags.

CPk: CA + packaging in polyethylene bags.

*Significant at 0.05 level; **Significant at 0.01 level; and nsNot significant at 0.05 level compared to controls.

Table 3. Effects of controlled atmosphere (CA) and CA in combination with Phaltan and polyethylene packaging on organic acids, amino acids, soluble proteins, and total proteins of lettuce.[a]

| Day of storage | Treat-ment | Organic acids mg/g | Amino acids mg/g | Soluble proteins mg/g | Total proteins g/100g |
|---|---|---|---|---|---|
| 0 | CR | 30.61 | 48.85 | 62.77 | 19.90 |
| 15 | CR | 33.67 | 49.97 | 63.54 | 21.17 |
| | CA | $31.69^{ns}$ | $44.97^{ns}$ | $54.20^{*}$ | $20.32^{ns}$ |
| | CPh | $27.69^{**}$ | $43.56^{**}$ | $62.86^{ns}$ | $20.13^{ns}$ |
| | CPP | $25.00^{**}$ | $40.71^{ns}$ | 49.09 | $20.92^{ns}$ |
| | CPk | $32.54^{ns}$ | $47.96^{ns}$ | $54.05^{*}$ | $21.06^{ns}$ |
| 30 | CR | 38.88 | 60.85 | 71.65 | 19.09 |
| | CA | $34.73^{ns}$ | $52.98^{**}$ | $68.86^{ns}$ | $19.74^{ns}$ |
| | CPh | $39.74^{ns}$ | $59.07^{ns}$ | $69.27^{ns}$ | $17.76^{ns}$ |
| | CPP | $37.49^{ns}$ | $57.37^{ns}$ | $70.47^{ns}$ | $18.61^{ns}$ |
| | CPk | $37.91^{ns}$ | $57.81^{ns}$ | $65.66^{ns}$ | $18.85^{ns}$ |
| 45 | CR | 35.49 | 73.62 | 64.14 | 21.93 |
| | CA | $34.26^{ns}$ | $96.25^{ns}$ | $55.82^{*}$ | $21.16^{ns}$ |
| | CPh | $39.14^{*}$ | $76.98^{ns}$ | $84.34^{**}$ | $19.78^{ns}$ |
| | CPP | $41.21^{**}$ | $81.04^{*}$ | $75.87^{**}$ | $21.71^{ns}$ |
| | CPk | $36.20^{ns}$ | $75.15^{ns}$ | $58.48^{ns}$ | $21.84^{ns}$ |
| 60 | CR | 34.78 | 77.48 | 64.29 | 19.34 |
| | CA | $35.88^{ns}$ | $79.04^{ns}$ | $53.79^{**}$ | $19.57^{ns}$ |
| | CPk | $36.93^{ns}$ | $82.13^{*}$ | $55.22^{**}$ | $19.01^{ns}$ |
| 75 | CR | 36.32 | 77.84 | 66.58 | 21.81 |
| | CA | $38.50^{ns}$ | $80.96^{ns}$ | $53.56^{**}$ | $22.31^{ns}$ |

a: Results expressed on dry weight basis.

CR: Conventional refrigeration; CA: Controlled atmosphere (2.5% $O_2$ and 2.5% $CO_2$).

CPh: CA + Phaltan (1,000 ppm). CPP: CA + Phaltan (1,000 ppm) + packaging in the polyethylene bags.

CPk: CA + packaging in polyethylene bags.

$^{*}$Significant at 0.05 level; $^{**}$Significant at 0.01 level; and $^{ns}$Not significant at 0.05 level compared to controls.

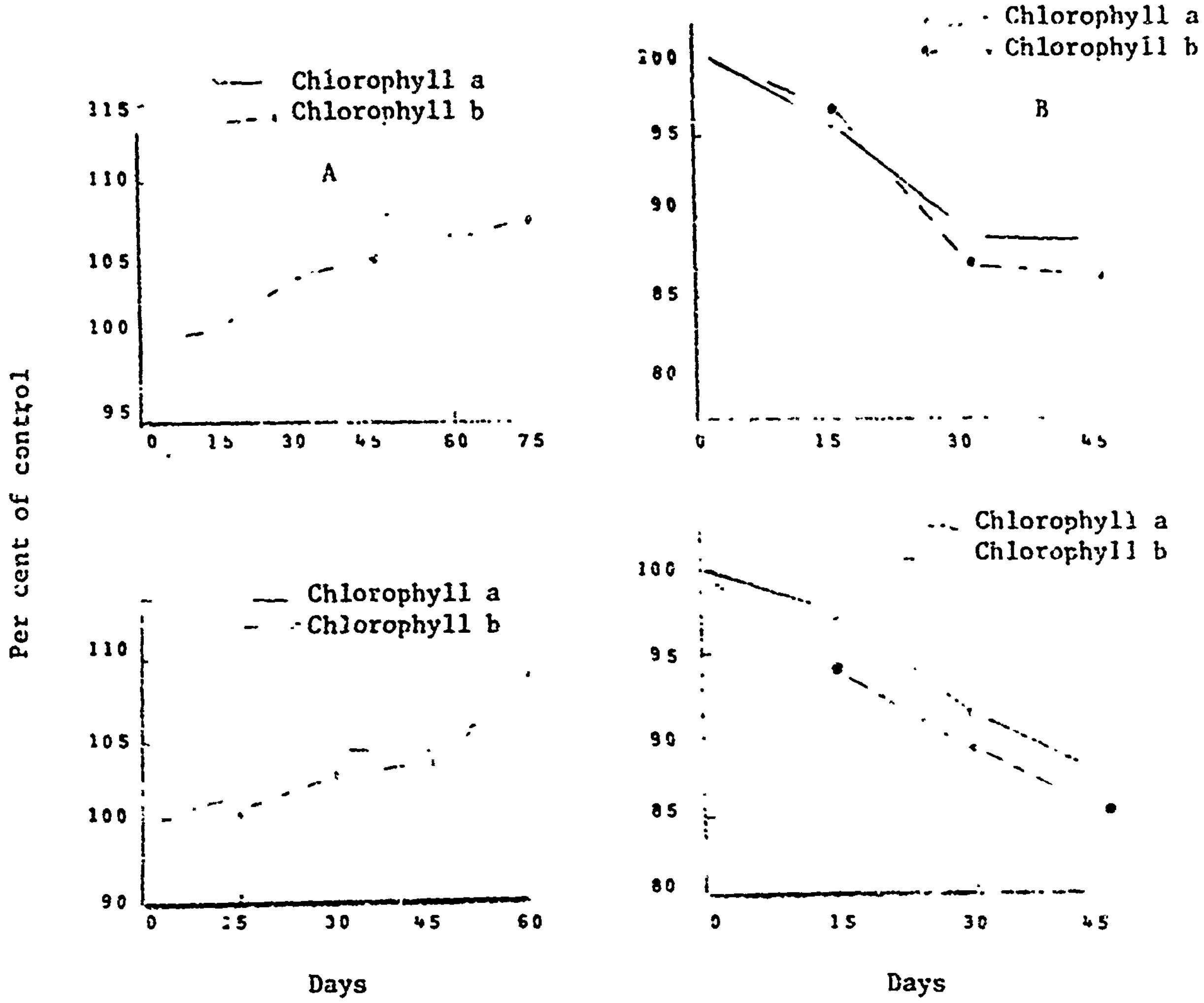


Fig. 1. Effects of controlled atmosphere (2.5 percent $O_2$, 2.5 percent $CO_2$) in combination with other treatments on the chlorophyll a and b of lettuce stored at 35° F. (A) CA, (B) CA + Phaltan (1,000 ppm), (C) CA + polyethylene packaging. (D) CA + Phaltan (1,000 ppm) + polyethylene packaging.

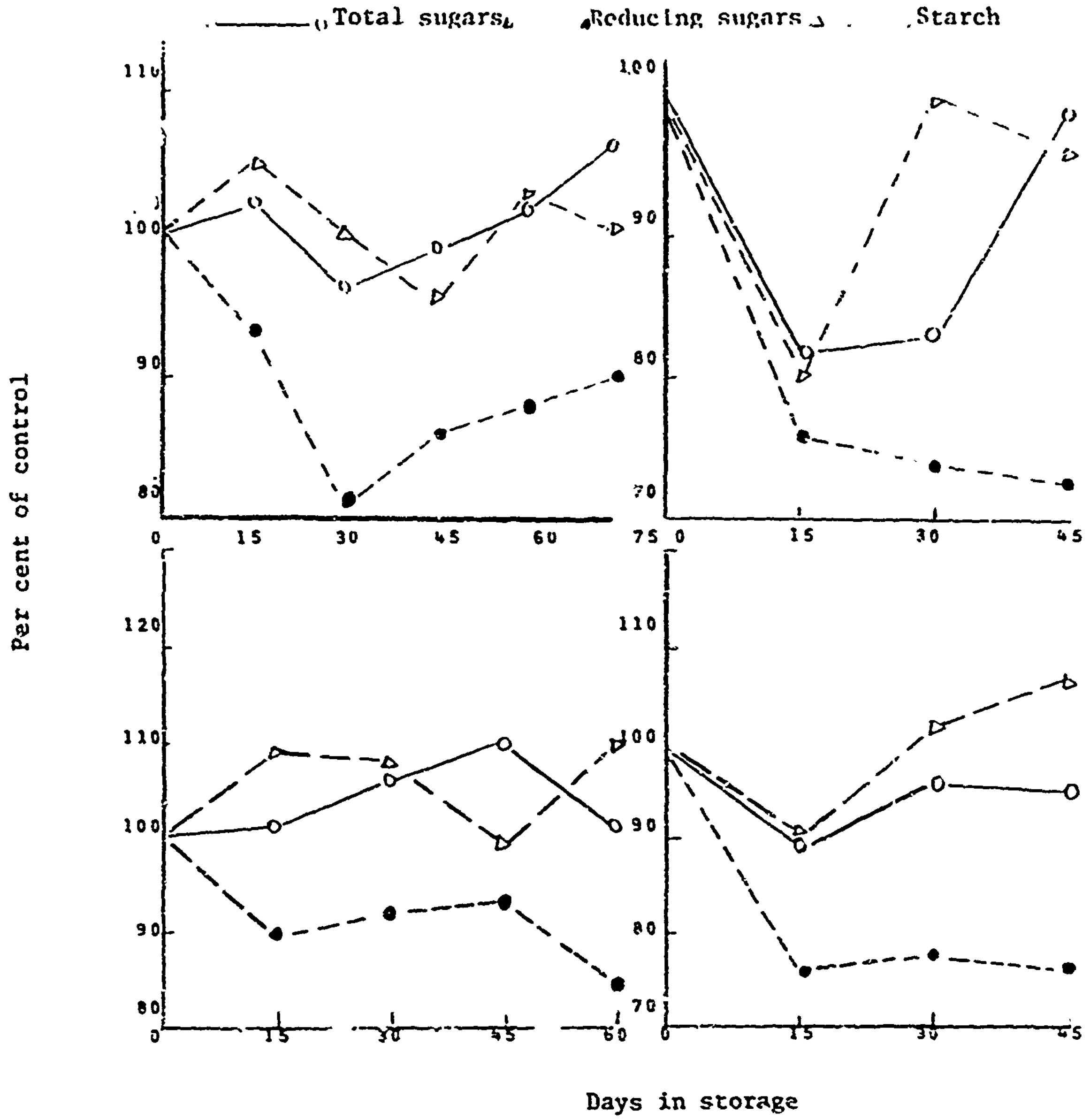


Fig. 2. Effects of controlled atmosphere in combination with other treatments on total sugars, reducing sugars, and starch. (A) CA, (B) CA + Phaltan, (C) CA + polyethylene packaging, (D) CA + Phaltan + polyethylene packaging.

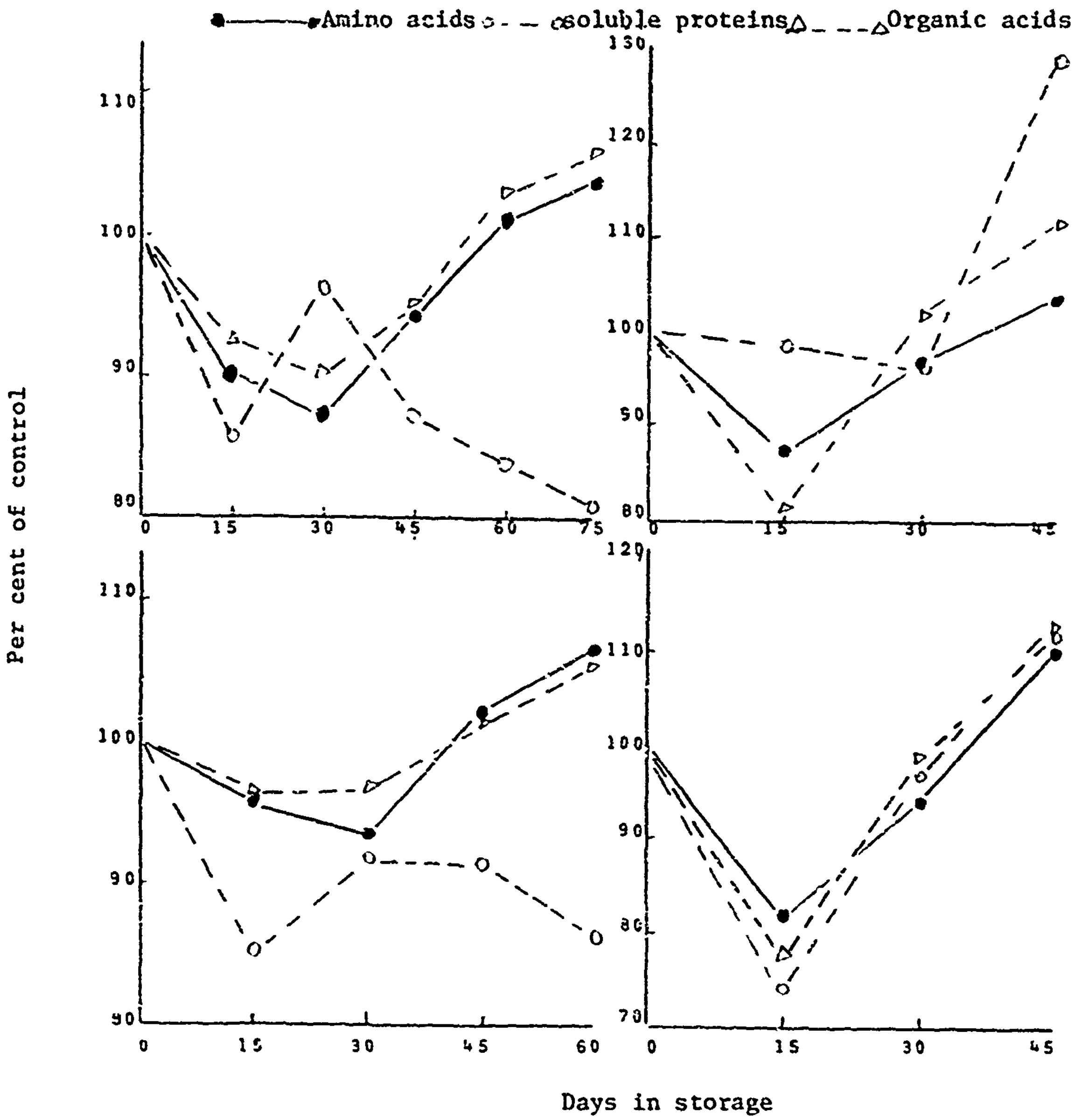


Fig. 3. Effects of controlled atmosphere in combination with other treatments on amino acids, soluble proteins, and organic acids of lettuce stored at 35° F. (A) CA, (B) CA + Phaltan, (C) CA + polyethylene packaging, (D) CA + Phaltan + polyethylene packaging.

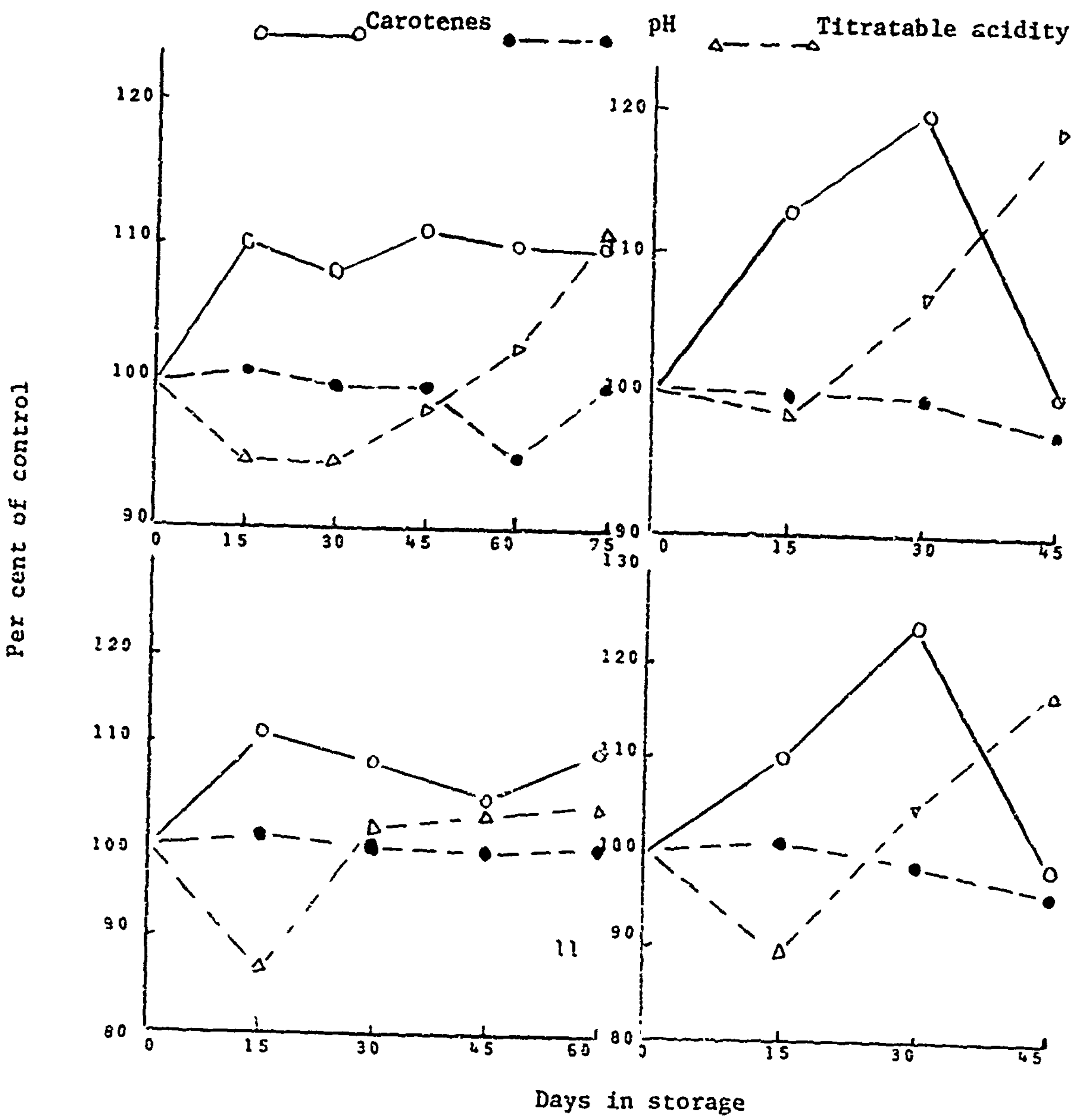


Fig. 4. Effects of controlled atmosphere in combination with other treatments on carotenes, pH, and titratable acidity. (A) CA, (B) CA + Phaltan, (C) CA + polyethylene packaging, (D) CA + Phaltan + polyethylene packaging.

## Summary

Lettuce, *Lactuca sativa* L., cultivar 'Great Lakes' was stored in a controlled atmosphere (CA consisting of 2.5% $CO_2$ and 2.5% $O_2$ at 35° F with or without pre-storage treatment with Phaltan® (1000 ppm) or polyethylene packaging). Samples were taken on the 15th, 30th, 45th, 60th, and 75th day of storage to determine the concentration of chlorophyll, pH, titratable acidity, organic acids total nitrogen, soluble proteins, free amino acids, total sugars, reducing sugars, and starch. CA significantly inhibited the degradation of chlorophyll throughout the storage period. The titratable acidity was lower in the lettuce stored in CA until the 45th day but was higher than the control on the subsequent days. There was no significant effect of CA or other treatments on total sugar, reducing sugar, starch, total N, pH, organic acids, amino acids, or total carotenes. The soluble proteins and the reducing sugars were lower in the CA lettuce than in the control lettuce. The lettuce treated with Phaltan® or Phaltan® in combination with polyethylene packaging had higher amounts of soluble proteins and lower amounts of chlorophylls.

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